

Assessment of Antioxidant Nutrient Intake and Status for Epidemiologic Research¹

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EXPANDED ABSTRACT

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Human studies that evaluate associations between antioxidant nutrients and risk of various chronic disease outcomes must assess exposure to target antioxidant nutrients. Exposure assessment can include questionnaire-based approaches to estimate intake of individual antioxidant nutrients, biomarker approaches to estimate individual antioxidant nutrient status in vivo, or combinations of methods. An alternative approach is to estimate the total antioxidant activity of the diet using biomarker approaches to estimate the antioxidant activity of foods. This article briefly reviews various approaches for assessing exposure to the following antioxidant nutrients for epidemiologic research: carotenoids, vitamin E, vitamin C, and selenium [reviewed in detail elsewhere (1)]. Biomarkers of oxidative stress status were covered elsewhere in this symposium.

The U.S. Department of Agriculture has released (2) and subsequently updated (3) a database for the carotenoid content of foods. This database allows investigators to estimate intake of the major dietary carotenoids (β -carotene, α -carotene, lycopene, lutein+zeaxanthin, β -cryptoxanthin) via the use of dietary questionnaires, an approach widely used in epidemiologic research. These same carotenoids (and others) also can be measured in tissues via the use of HPLC, which has the additional advantage of being able to separate various carotenoid geometric isomers (of potential relevance to chronic disease etiology). The biomarker (HPLC) approach also assesses internal concentrations, which are affected greatly by carotenoid bioavailability, known to vary considerably in different foods. The disadvantages of current biomarker

(HPLC-based) methods for carotenoid assessment in epidemiologic research include cost (typically \$50–\$100/assay) and the need for an invasive procedure to obtain tissue for analysis (e.g., venipuncture, biopsy). Recognizing these limitations, existing technologies now are being developed and validated for the noninvasive, cost-effective assessment of carotenoids in vivo. Emerging methods include skin reflection spectrophotometry (4) and Raman resonance spectroscopy (5,6). These methodologies have not yet been sufficiently validated for use in epidemiologic research, but validation studies are underway, including one such study in our research group.

Exposure assessment for tocopherols, like carotenoids, is complicated by the fact that multiple chemical substances are involved (e.g., α -, β -, γ -, δ -tocopherol; 4 tocotrienols). Current U.S. nutrient databases estimate the vitamin E content of foods based on contributions of the various forms of vitamin E, expressed as α -tocopherol equivalents (based on the rat fetal resorption assay). However, the Institute of Medicine recently concluded that the various forms of vitamin E are not interconvertible in humans, with vitamin E requirements now based solely on the 2R-stereoisomeric form of α -tocopherol (7). The dynamic nature of vitamin E equivalencies suggests that food databases should specifically separate out the various tocopherols/tocotrienols, both for etiologic research and to allow for possible future revisions to vitamin E equivalencies.

Given these and other difficulties [see (1)] in assessing vitamin E intake from foods, biochemical assessment of various tocopherols in biological samples is considered the preferred method for assessment and is an approach that has been utilized widely. HPLC methods allow for the simultaneous measurement of various tocopherols, particularly α - and γ -tocopherol. Of note, the α -/ γ -tocopherol ratio has been suggested as a biomarker for the use of supplemental vitamin E (8).

Vitamin C can be assessed by either dietary or biochemical approaches, both of which have advantages and disadvantages. Intake assessment is inexpensive, although measurement error is inevitable due to variable loss of vitamin C in foods during food handling and cooking. Plasma ascorbate can be measured reasonably as a biomarker using HPLC methods; however,

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fasting generally is advised, and samples require acid preservation at the time of collection. As is the case with many nutrients, plasma ascorbate concentrations are modestly correlated with dietary intake, with plasma concentrations saturating at higher intake levels (9), thus better representing internal exposures.

The selenium content of foods varies, depending on the selenium content of the soil where plants are grown or where animals consume forage crops. Similar food items can have 10-fold differences in selenium content, making dietary assessment of selenium intake from foods unreliable. Biomarkers thus are preferred for assessing selenium exposure. Some approaches used include plasma and/or toenail selenium concentrations. Toenail selenium has been shown to reflect intake over a period of 26–52 wk or more (10).

Biomarker approaches thus currently exist for all of these major classes of antioxidant nutrients. Once individual antioxidant nutrient exposures are estimated for research, a current challenge in the field is the need to develop and validate methodology to derive exposure estimates that simultaneously consider multiple antioxidant nutrients (e.g., antioxidant indices). That is, one's overall antioxidant nutrient status might be of greater relevance for chronic disease risk than individual antioxidant nutrient levels, given the known biochemical interactions of antioxidant nutrients (11,12). This suggests the need to develop more comprehensive indices of antioxidant nutrient intake/status. One approach includes stratification of subjects by more than one antioxidant nutrient (e.g., low in selenium and low in vitamin E). Another involves creating score variables for various antioxidant nutrients (e.g., assign 3 points for subjects in the highest tertile of an antioxidant nutrient, 2 points for those in the middle tertile, 1 point for the lowest tertile; sum overall antioxidant nutrient exposures), while another involves the creation of indices based on statistical models. For example, we have used principal component analyses to develop an antioxidant nutrient index that simultaneously considers intake of several antioxidant nutrients [e.g., carotenoids, flavonoids, tocopherols, selenium, vitamin C (13)].

The previously described methods implicitly assume that the antioxidant properties of foods reflect the antioxidant nutrient content of those same foods. However, more direct evaluation of the antioxidant capacity of various food items also is being done, for example, using the oxygen radical absorbance capacity (ORAC) and the ferric reducing antioxidant power (FRAP) assays to test various foods (14). Antioxidant food scores based upon biochemical assessments have the advantage of partially allowing for synergism of antioxidants found in the same food item—partial because lipid-soluble and water-soluble extracts typically are evaluated separately for antioxidant capacity. However, a substantial limitation of this approach is that some antioxidants that contribute to antioxidant activity *in vitro* may be poorly absorbed *in vivo*. Also, such databases are incomplete at

present, limiting their potential usefulness for epidemiologic research.

Epidemiologic studies will continue to contribute to our understanding of the pros and cons of antioxidant nutrients with regard to chronic disease risk; however, increased attention to valid methods for assessing exposures is needed to move the field forward. In particular, there is a critical need to develop and validate approaches for assessing antioxidant nutrient status *in vivo* and antioxidant status of the diet more globally, rather than on a nutrient-specific basis. Improved exposure assessment, along with improved assessment of biomarkers of oxidative stress (covered elsewhere in this symposium), should contribute to a clearer understanding of antioxidants and human disease.

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